flagellum (wavelength and amplitude), effect swimming characteristics. MATLAB tracking and analysis algorithms are used to extract motility parameter quantities.

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Helical Flow of Surface Protein required for Bacterial Locomotion

Daisuke Nakane[1], Keiko Sato[1], Hirofumi Wada[2], Mark J. McBride[3], Koji Nakayama[1].
[1]Nagasaki University, Nagasaki, Japan, [2]Ritsumeikan University, Shiga, Japan, [3]University of Wisconsin-Milwaukee, Milwaukee, WI, USA.

Cells of *Flavobacterium johnsoniae* and of many other members of the phylum *Bacteroidetes* exhibit rapid gliding motility over surfaces by a unique mechanism. These cells do not have flagella or pili, and instead rely on a novel motility apparatus comprised of Gld and Spr proteins. SprB, a 669 kDa cell-surface adhesion, is required for efficient gliding. SprB was visualized by electron microscopy as thin 150 nm long filaments extending from the cell surface. Fluorescence microscopy revealed movement of SprB proteins toward the poles of the cell at approximately 2 μm/s. The fluorescent signals appeared to migrate around the pole and continue at the same speed toward the opposite pole along an apparent right-handed helical closed loop. Movement of SprB, and of cells, was rapidly and reversibly blocked by the addition of CCCP, which dissipates the proton gradient across the cytoplasmic membrane. In a gliding cell, some of the SprB protein appeared to attach to the substratum. The cell body moved forward and rotated with respect to this point of attachment. Upon reaching the rear of the cell, the attached SprB was often released from the substratum, and apparently recirculated to the front of the cell along a helical path. The results suggest a model for *Flavobacterium* gliding, supported by mathematical analysis, in which adhesins such as SprB are propelled along a closed helical loop track, generating rotation and translation of the cell body.

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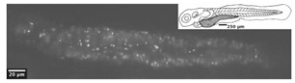
Imaging Colonization Dynamics and Rheological Properties of a Host and its Developing Microbiome by Light Sheet Microscopy

Michael J. Taormina, Matthew Jemielita, Zac Stephens, Adam Burns, Jennifer Hampton, Karen Guillemin, Raghuveer Parthasarathy.
University of Oregon, Eugene, OR, USA.

For microbes colonizing an animal host, the mechanical properties of the host environment are of great importance, affecting motility and therefore (presumably) the ability to establish a stable population. Indeed, some species possess the ability to affect the fluidity of their environment, both directly by chemically modifying it, and indirectly by influencing the host's production of secretory cells. By utilizing the unique strengths of light sheet fluorescence microscopy combined with the techniques of microrheology, we can witness early encounters between colonizing bacteria and an initially germ-free host, and directly measure the material properties of the intestinal environment. We performed three-dimensional imaging of the entire larval zebrafish gut for twenty-four hours following bacterial inoculation, yielding highly resolved spatiotemporal information about the interplay between microbes and host. Additionally, by driving magnetically doped micron-scale probes, the rheology of the mucosal layer within the fish can be measured over three decades of frequency, adding physical knowledge of the environment to quantitative observations of a complex biological system's maturation.



3281-Pos Board B436

Exploration of Bdellovibrio Chemotaxis and Predation using Microfluidics

Emma Garst[1], Veronika Kivensen[1], Emma Hughes[1], Jessica McKenzie[1], Anne Murdaugh[2], Eileen Spain[3], Megan Ferguson[4], Megan E. Nunez[1].
[1]Mount Holyoke College, South Hadley, MA, USA, [2]Rollins College, Winter Park, FL, USA, [3]Occidental College, Los Angeles, CA, USA, [4]SUNY New Paltz, New Paltz, MA, USA.

Bdellovibrio bacteriovorus is a predatory, gram-negative bacterium that preys on other gram-negative bacteria. It has long been hypothesized that B. bacteriovorus can sense prey in the environment and move toward them, and recent genomic sequencing indicates that B. bacteriovorus has approximately 20 methyl-accepting chemotaxis receptor proteins and the full flagellar machinery necessary for chemotaxis. Nonetheless, B. bacteriovorus chemotaxis has never been demonstrated in the laboratory. As a result, the molecules it might use to target and track its prey have not been identified. A road block to prior research has been the limitations on traditional chemotaxis assays; B. bacteriovorus does not form colonies on agar media plates and it has been known to move up to 100 body lengths per second, which makes it difficult to track its growth or movement in response to a specific chemoeffector. To address these issues, we have designed a microfluidic device to measure the reaction of B. bacteriovorus to specific chemoeffectors. The small dimensions and controlled flow in a microfluidic device allow us to introduce B. bacteriovorus to a gradient of chemoeffectors such as sugars, metabolites, and signaling molecules. With multiple outlets containing a range of chemoeffector concentrations, we can observe both attractive and repellent responses, as well as score the degree to which B. bacteriovorus reacts to these chemicals. Thus a microfluidic device provides significant advances over classic ''on/off'' chemotaxis assays, allowing us to explore for the first time the target molecules and affinity of B. bacteriovorus chemotaxis receptors.

3282-Pos Board B437

On Time Reversal Symmetry and Bacterial Chemotaxis

Xiao-Lun Wu, Altindal Tuba.
University of Pittsburgh, Pittsburgh, PA, USA.

Motility of polar flagellated bacteria is typically forward and backward in rapid succession. We recently found that one of the marine species, Vibrio alginolyticus, incorporates a flick movement at the end of the backward swimming interval, breaking the time reversal symmetry of the swimming trajectory. A flick in this bacterium is functionally equivalent to a tumble of peritrichously flagellated bacteria, such as Escherichia coli, causing the cell body to deflect in a new direction before the next run starts. Since V. alginolyticus is capable of swimming in both forward and backward directions, it raises an interesting question about how the chemotaxis behavior of this bacterium is regulated. Herein, we provide experimental evidence showing that the marine bacterium differentiates chemical signals detected in the two swimming intervals and responds in the manner that is consistent with the chemotaxis strategy where the forward swimming interval is exploratory and the backward interval is exploitative.

3283-Pos Board B438

Chemotactic Response of Escherichia Coli to Repellents, CoCl2 and NiCl2

R.V.S. Uday Bhaskar, Rajesh S. Jesudasan, Deepti Deepika, Mahesh S. Tirumkudulu, K.V. Venkatesh.
IIT Bombay, mumbai, India.

Negative chemotaxis refers to the motion of microorganisms away from harmful chemicals. Soft agarose gel assay has been traditionally used to characterize the response to various repellents. In this study, we use the ''chemical-in-plug'' method to quantify the motion of Escherichia coli in the presence of repellents, NiCl2 and CoCl2, over a broad range of concentrations. These experiments were complimented with drift velocity measurements of individual bacteria in controlled gradients using a capillary assay. The latter also revealed the tumbling frequency and steady state clockwise bias for varying concentrations of repellents thereby providing insight into adaptation to repellents. The experimental technique yielded the motion of the bacteria in space and time and further related the motion to the evolving concentration profile of the repellent. Results show that the bacteria exhibit logarithmic sensing to the repellents, i.e., the drift velocity of E.coli is proportional to the logarithmic concentration gradient suggesting Weber law. The predictions of a standard population based model agreed with the observed linear behavior when the binding of the repellent to the receptor was sub-sensitive. This was borne out by a low value of Hill coefficient ($n<<1$) used to describe the binding characteristics of the receptors. The analysis shows that the binding characteristics for the repellents was sub-sensitive in contrast to an ultra-sensitive response observed for attractants suggesting a negative cooperative behavior of receptors. The above experiments suggest that negative cooperativity allows the cells to respond to harmful chemicals without saturation even at high concentration.

3284-Pos Board B439

Role of the Pseudomonas Aeruginosa Flagellar Motor in Swimming Motility and Chemotaxis

Chui Ching Wong, Chen Qian, **Keng-Hwee Chiam**.
National University of Singapore, Singapore, Singapore.

Flagellar-driven swimming motility is well-established in some bacterial model organisms, and it is best described in the case of Escherichia coli. However, increasing genetic and structural data show that diversity in flagellar motors exists across the bacterial kingdom, where new paradigms of swimming motility may be discovered. In this report, we describe the flagellar motor function of monotrichous P. aeruginosa, and show that unlike E. coli, it is a motor that rotates in both counter-clockwise (CCW) and clockwise (CW) directions giving rise to a 'run-and-reverse' trajectory. Additionally, the flagellar motor exhibits multiple speeds in the CCW but not the CW direction. using a microfluidic-based assay, we show that in the presence of a chemoattractant (serine), the cells alter their run-length, switching frequency and motor speeds in order to move toward favorable environments. Therefore, in chemotaxis, apart from varying the switch frequency, the P. aeruginosa flagellar motor has an